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GlobalFiler™
Powered by 6-Dye™

Internal Validation of the GlobalFiler STR Kit

24th International Symposium

On

Human Identification

Atlanta, Georgia 2013



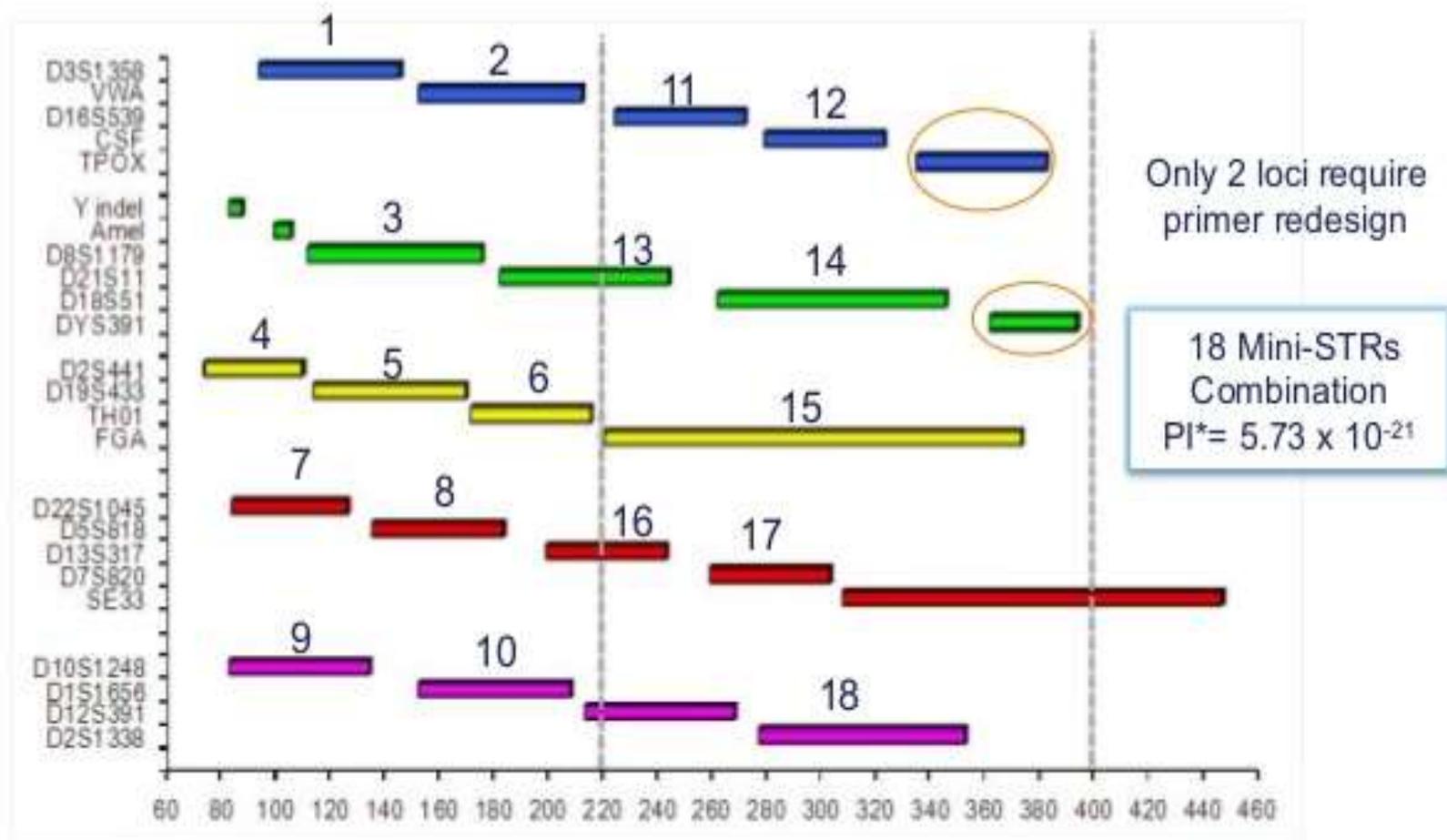
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Alabama Department of Forensic Sciences

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The GlobalFiler™ Kits: 6-Dye Configuration



GlobalFiler™ Kit
10 Mini-STRs
PI* = 9.2×10^{-12}

MiniFiler™ Kit
8 Mini-STRs
PI* = 1.05×10^{-10}

97% of alleles in
allelic ladder <400bp

FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories

8.3.1 For Internal Validation Studies:

- a. Have internal validation studies been documented and summarized?
- b. Have all internal validation studies conducted on or after July 1, 2009, included, as applicable:



1. Known and non probative evidence samples or mock evidence samples?

Yes No N/A

2. Reproducibility and precision?

Yes No N/A

3. Sensitivity and stochastic studies?

Yes No N/A

4. Mixture studies?

Yes No N/A

5. Contamination assessment?

Yes No N/A

Internal Validation Studies Background

Current Test Kit:	Identifiler STR Kit
Detection Platform:	3130 Genetic Analyzer
3130 Analytical Threshold:	75 rfu



Test Kit:	GlobalFiler STR Kit
Detection Platform:	3500 Genetic Analyzer (8 capillary)
3500 Analytical Threshold:	175 rfu – starting point



Known and Non-Probative Evidence Samples

Previously extracted non-probative evidence samples, which mimic other sample types routinely encountered in forensic casework, were tested with the GlobalFiler STR Kit. The results obtained using GlobalFiler were compared to those previously obtained with the Identifiler STR Kit.

The sample types included in this study included:

- sexual assault samples (VS, condom, bedding, and panties)**
- wearer items**
- cigarette butts**
- bottle/can swabs**
- blood swabs**
- degraded/inhibited samples**
- reference samples (buccal swabs and blood cards)**



Known and Non-Probative Evidence Samples

GlobalFiler:

- tests 24 loci
- 21 loci are autosomal STRs
- *12 loci < 250 bp*

Identifiler:

- tests 16 loci
- 15 loci are autosomal STRs
- *9 loci < 250 bp*

variables:

- input volume differences (*10ul vs 15ul*)
- chemistry differences between the GF and ID kits?
- 3500 and 3130 differences?
 - injection parameters
 - analysis algorithms

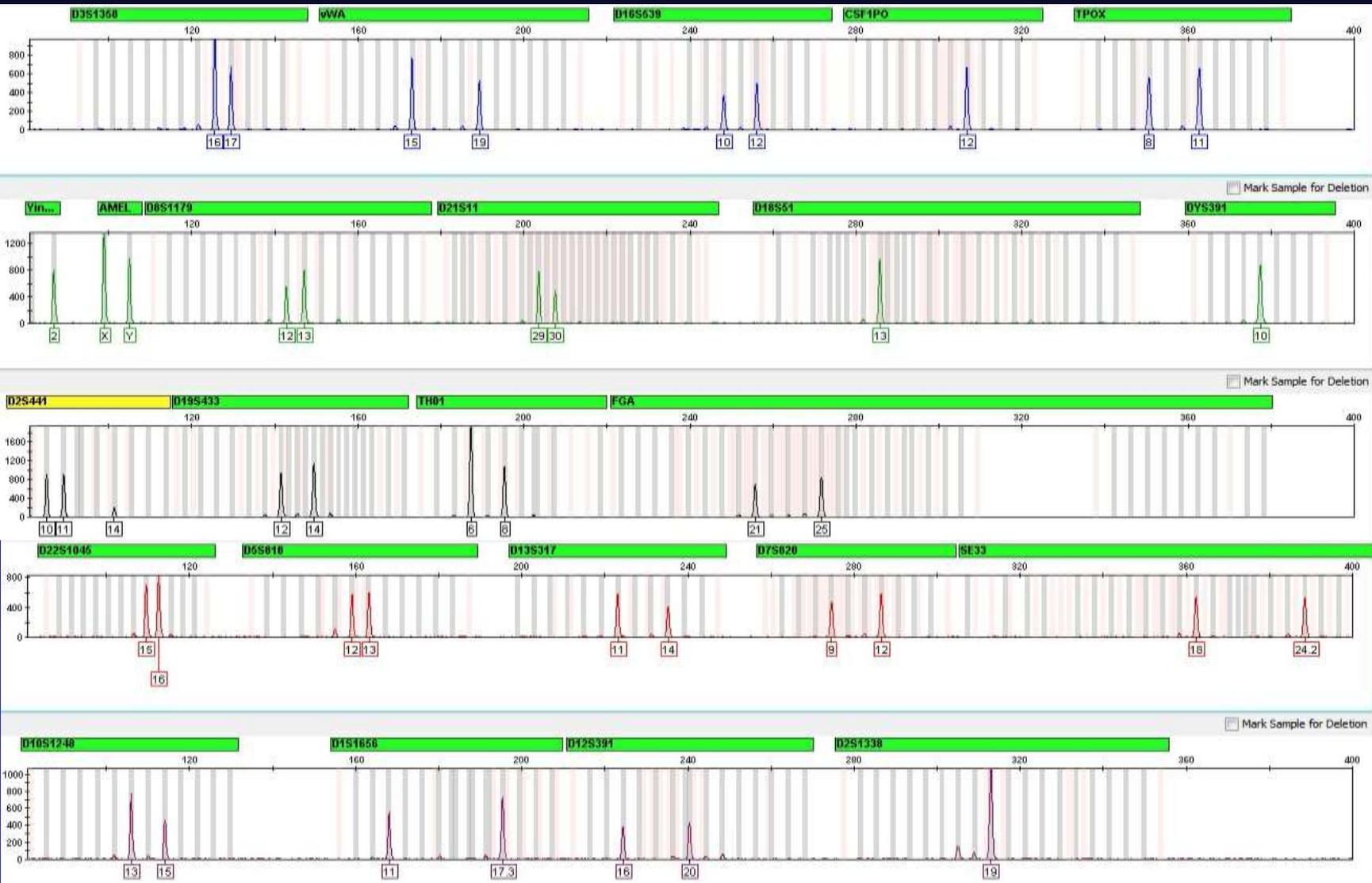


- Good concordance between GlobalFiler and Identifiler
- More information from GlobalFiler due to the additional dye and the number of loci < 250 bp
- GlobalFiler more effective in overcoming **inhibition and degradation**

1) Semen stain from a quilt

- 0.041 ng/ μ l
- 0.41 ng amplified with Identifiler
- maximum injection parameters on the 3130 (2 μ l/ 9 secs)
- partial male DNA profile
(10 loci of data with 7 of the CODIS core)

GlobalFiler



- 0.62 ng amplified with GlobalFiler
- standard injection parameters on the 3500 (1 μ l/15 secs)
- full male DNA profile (13 CODIS core)



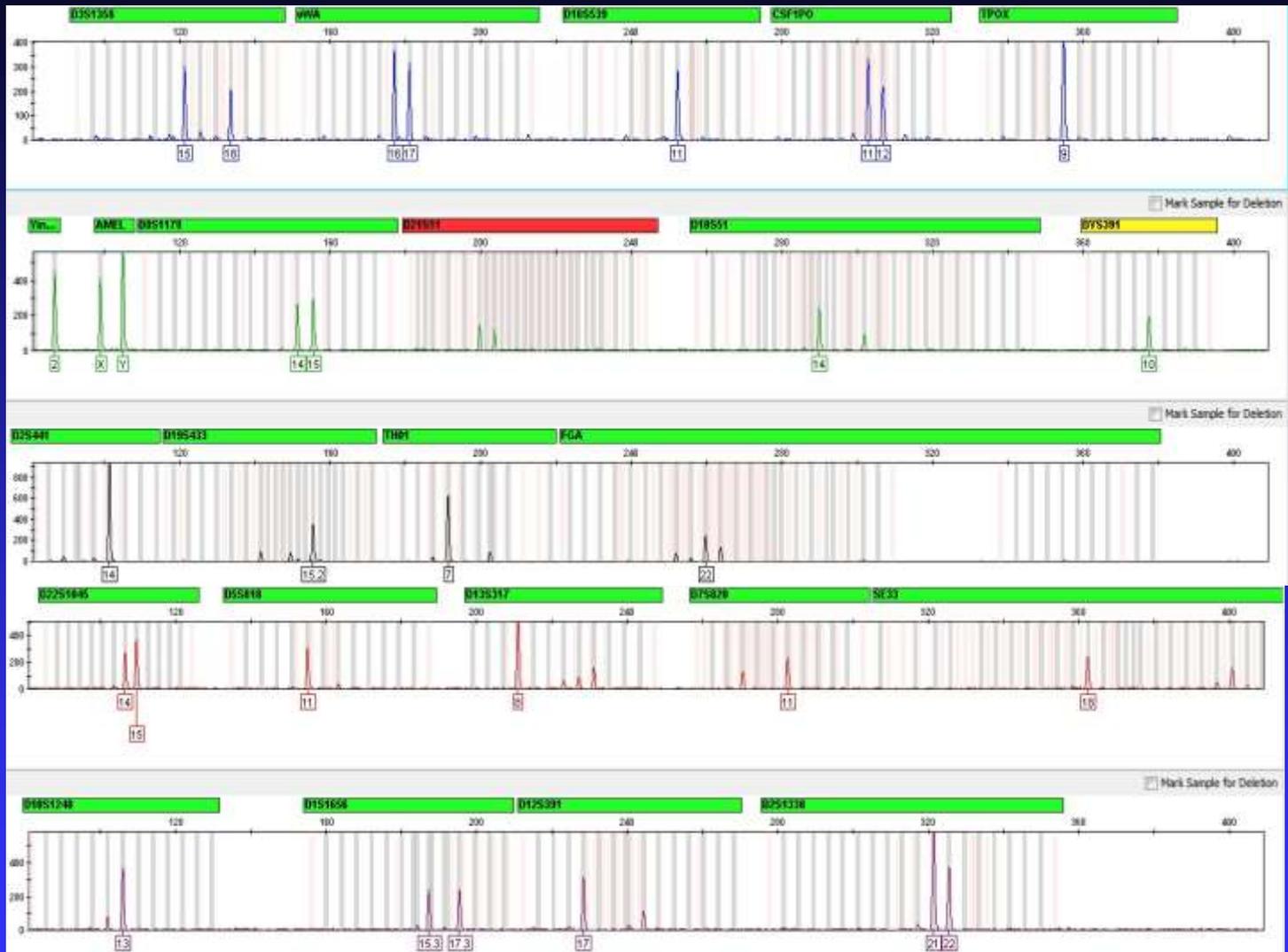
Known and Non-Probative Evidence Samples

2) Semen stain from a pair of panties

- 0.0091 ng/ μ l
- 0.09 ng amplified with Identifiler
- maximum injection parameters on the 3130 (2 μ l/ 9 secs)
- partial male DNA profile
- 12 loci of data with 9 of the CODIS core



GlobalFiler



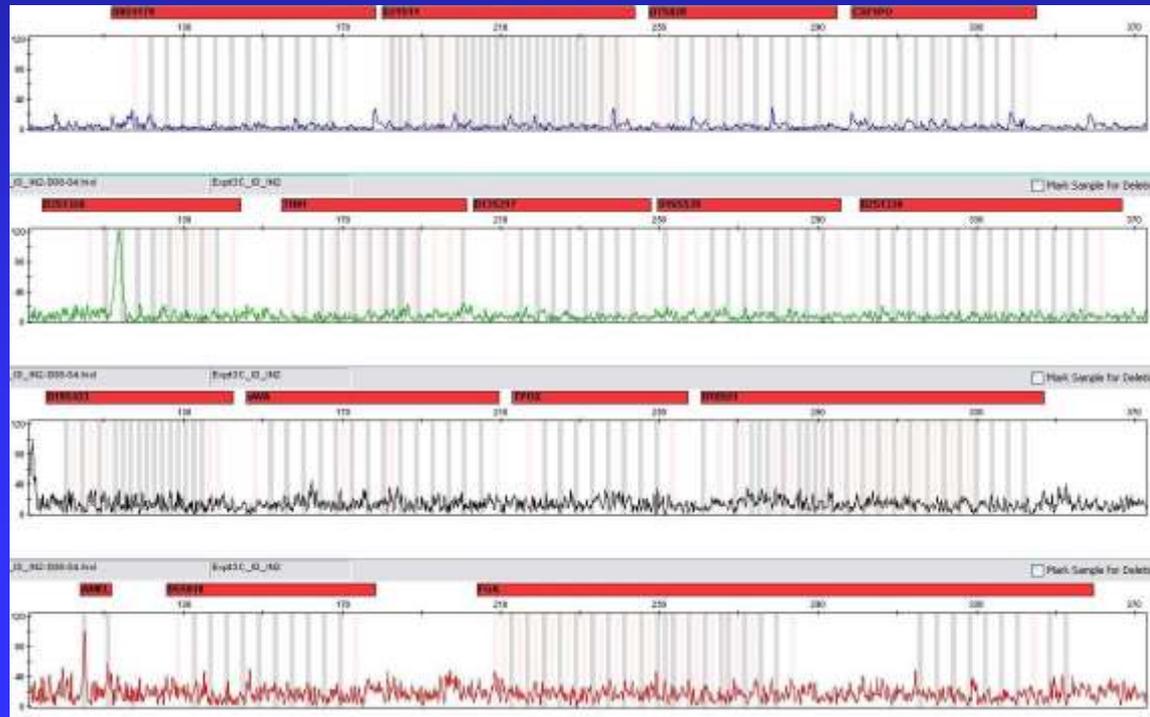
- 0.14 ng amplified with GlobalFiler
- standard injection parameters on the 3500 (1 μ l/15 secs)
- partial male DNA profile (**23 loci of data and 12 CODIS core**)



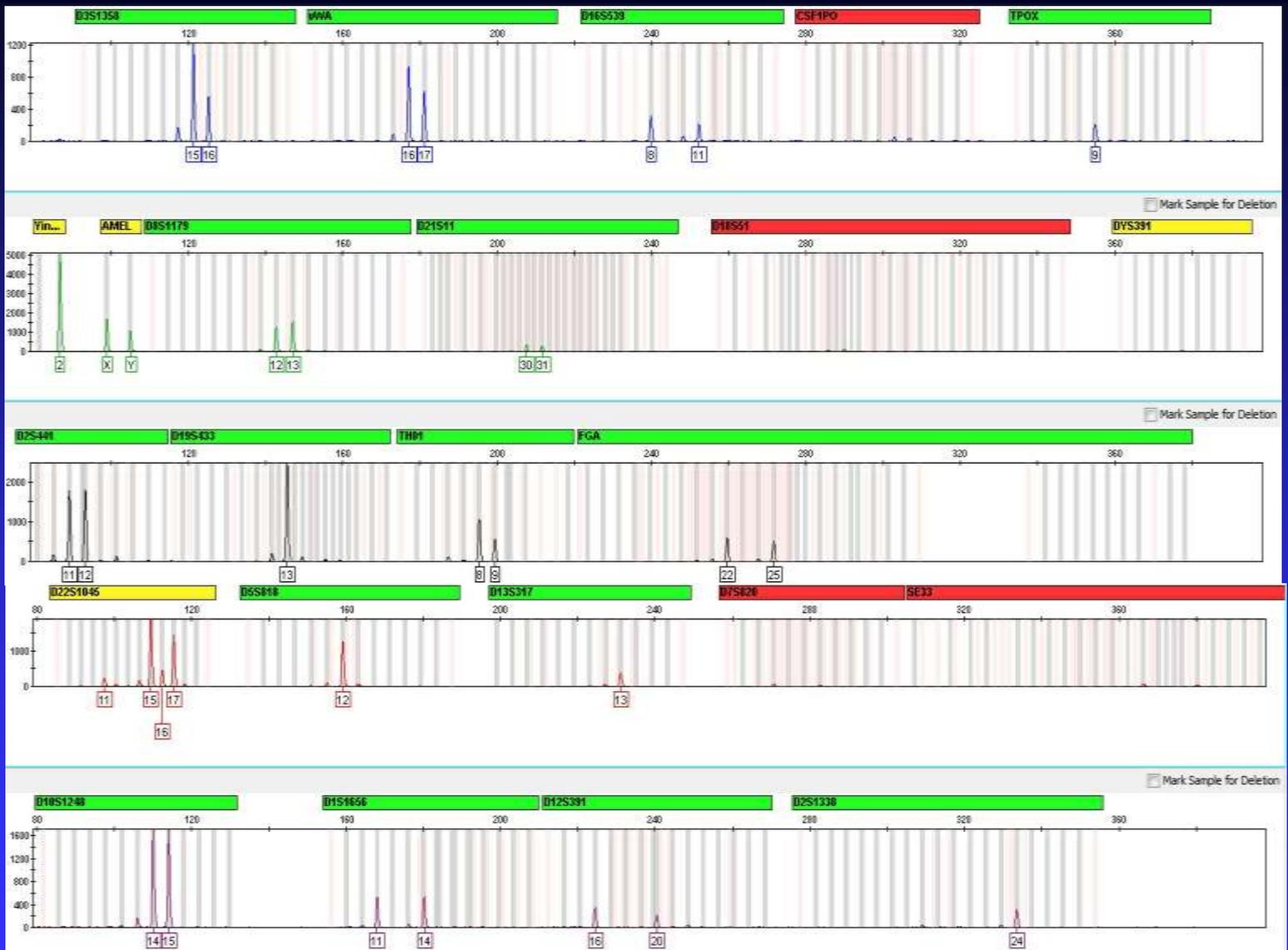
Known and Non-Probative Evidence Samples

3) Swabbing of a concrete block

- 0.17 ng/ μ l
- 1 ng amplified with Identifiler
- standard injection on the 3500 (1 μ l/15 secs)
- *no results*
- *inhibition suspected due to extract color*



GlobalFiler



- 1 ng amplified with GlobalFiler
- standard injection parameters on the 3500 (1 μ l/15 secs)
- **partial DNA profile (19 loci of data; 10 CODIS core)**

Reproducibility, Precision, and Accuracy

Precision: characterizes the amount of agreement among a series of individual (or repeated) measurements

-**Precision** does not relate to the accuracy of the measurements

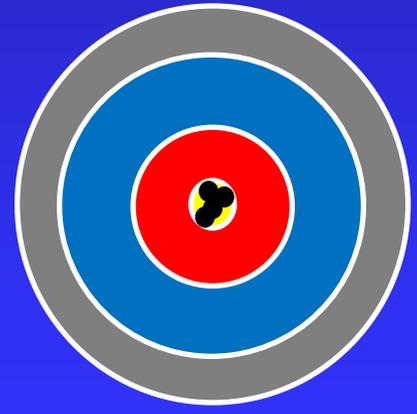
Accuracy: describes how close the measured values are to the true value



Low Precision
Low Accuracy



High Precision
Low Accuracy



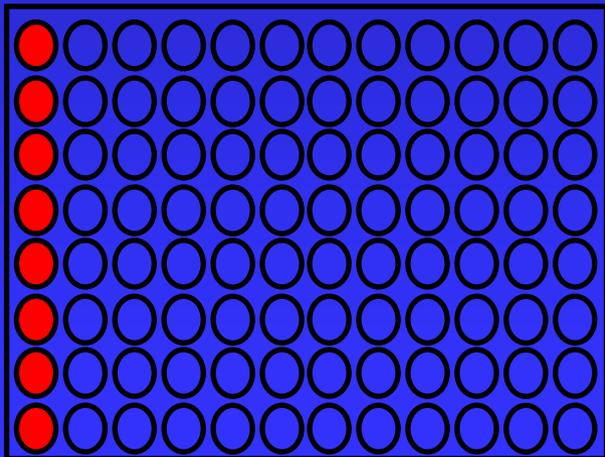
High Precision
High Accuracy

Reproducibility and Precision Study Design

GlobalFiler allelic ladder was prepared in one column and injected eight (8) times on the 3500 Genetic Analyzer

-size determinations were conducted on all alleles of the ladder for each injection

-standard deviations in base pair sizing were calculated for each allele in the ladder

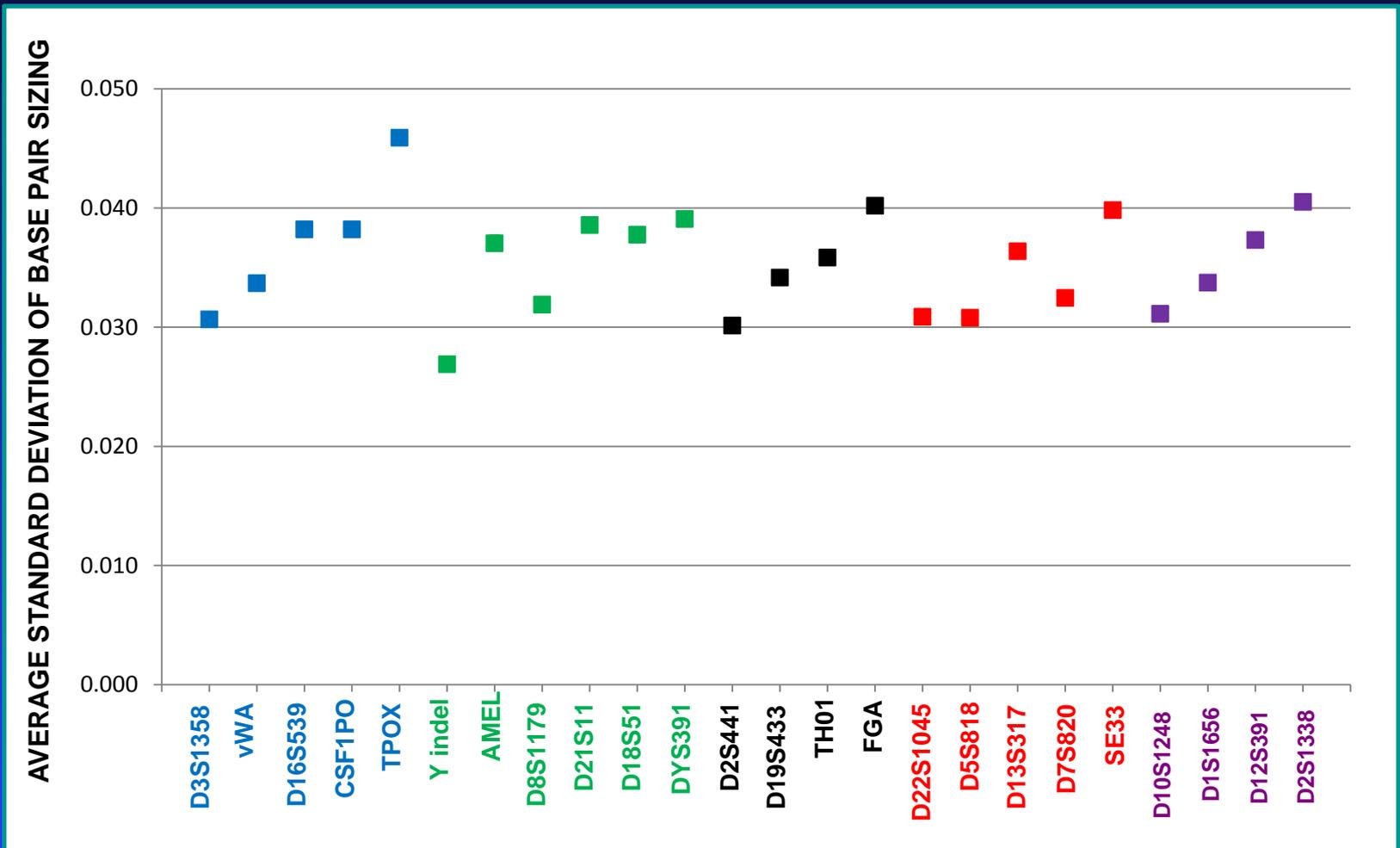


GlobalFiler allelic ladder – 343 alleles across
24 loci

↓
2,744 alleles per 8-cap injection

↓
21,952 alleles per run of 8 injections

Reproducibility and Precision of GlobalFiler Allelic Ladder



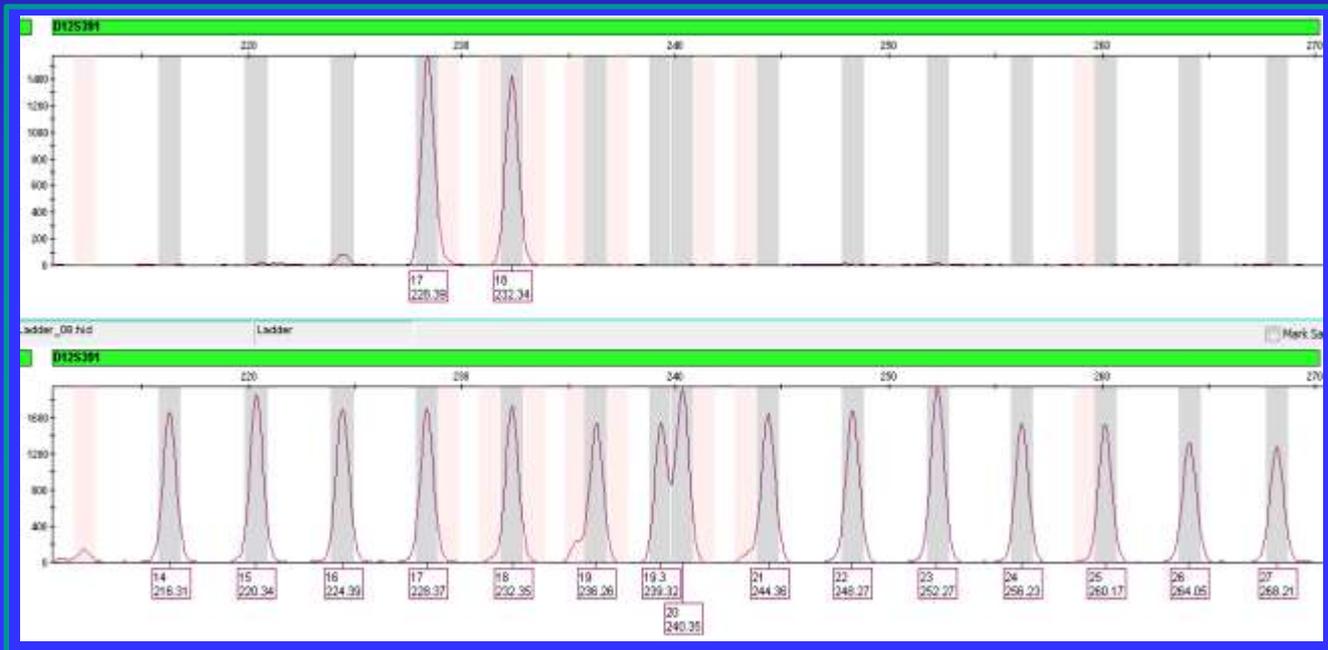
-standard deviation was averaged across all the alleles at each locus

Accuracy Study Design

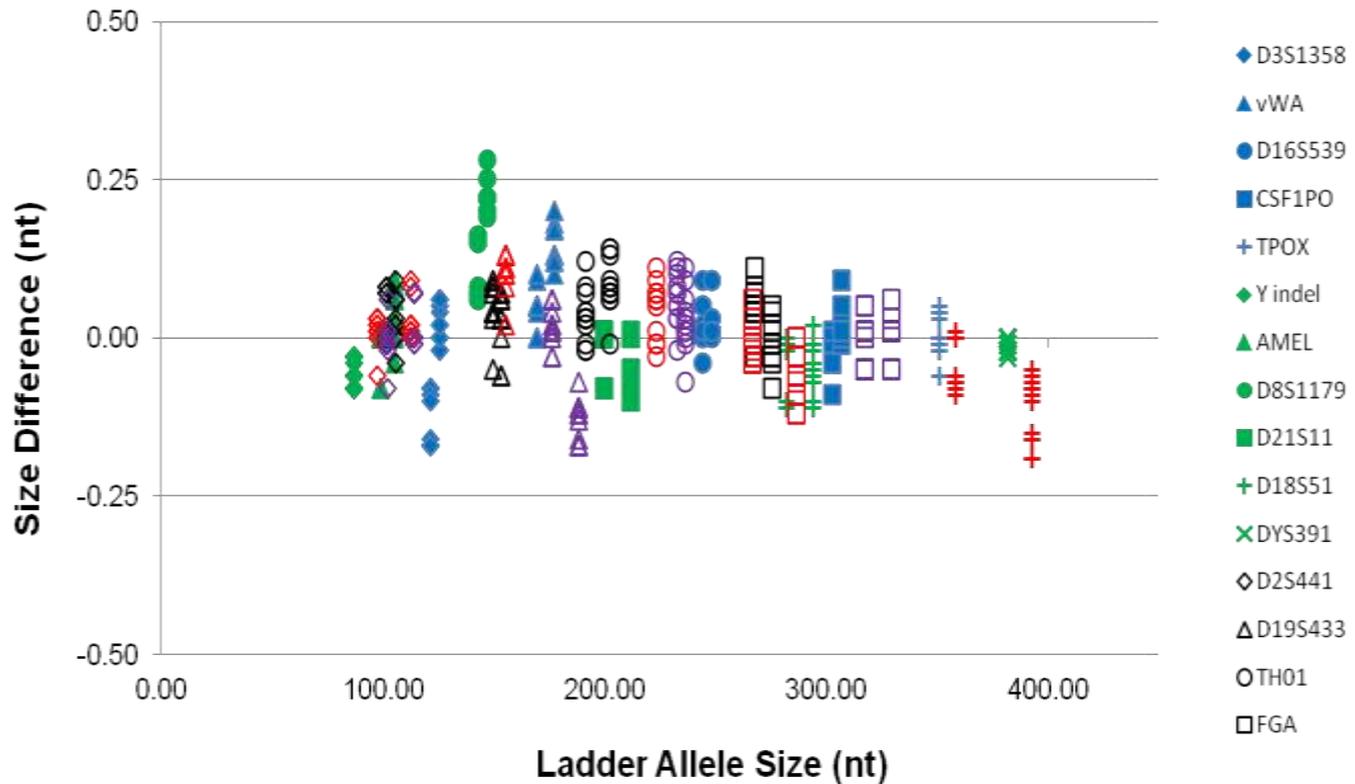
One (1) ng of the 007 Human DNA control was amplified concurrently four times with each amplified product prepared for electrophoresis in triplicate for a total of twelve (12) samples. The twelve (12) samples were injected on three separate days generating data for thirty-six (36) samples across three separate runs.

Accuracy: describes how close the measured values are to the true value

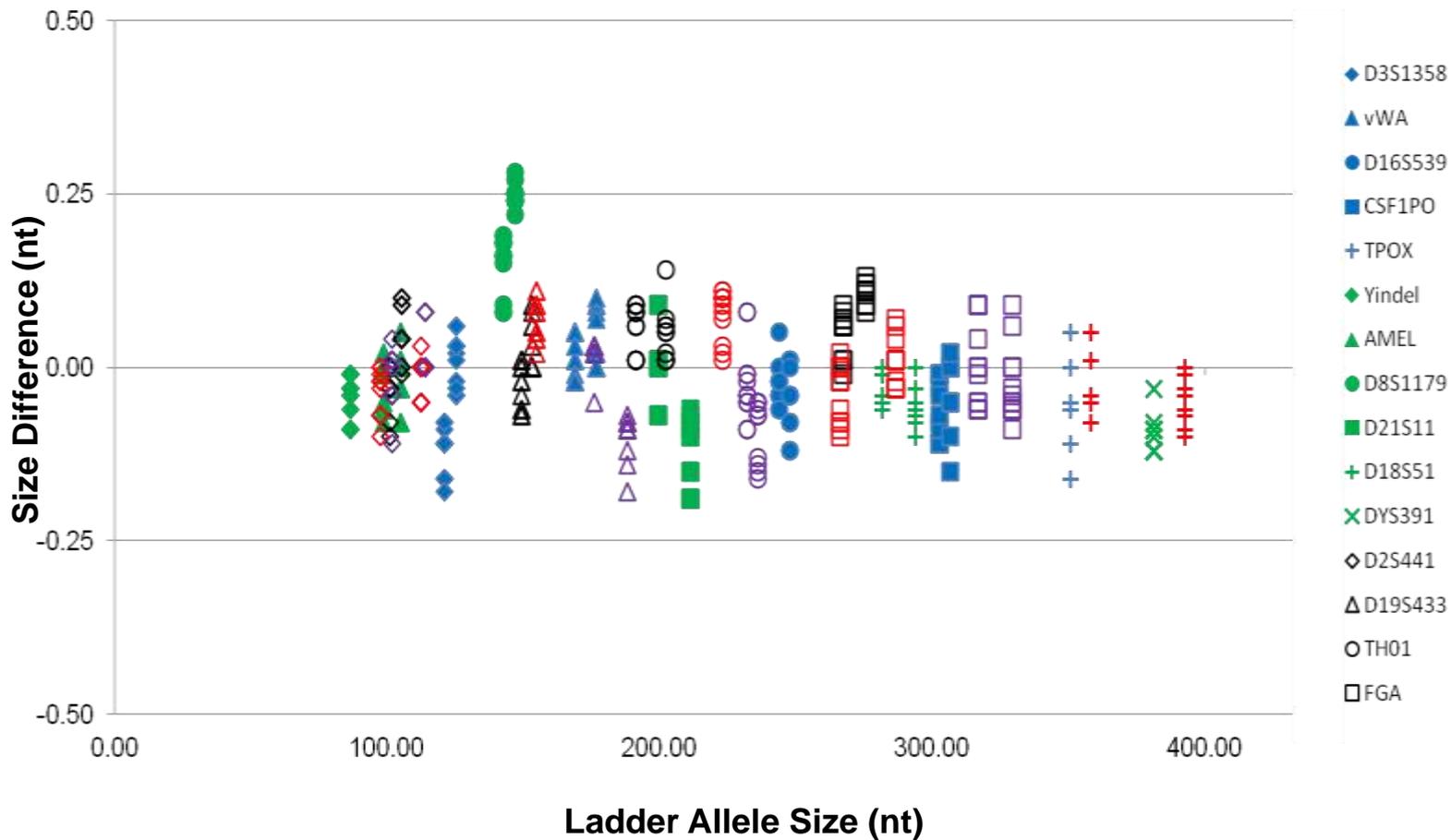
The measurement of each allele in the 007 sample was compared to the measurement of the corresponding allele in the GlobalFiler allelic ladder



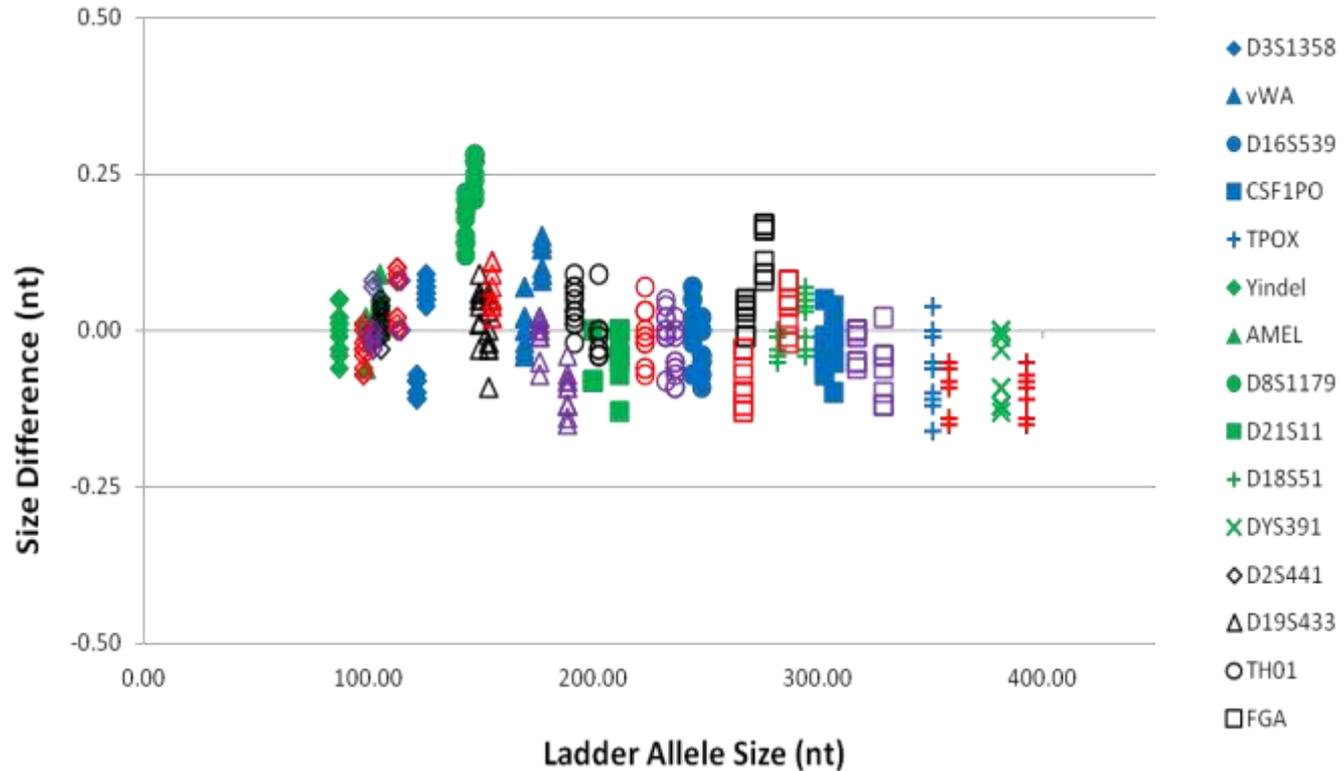
Accuracy Study Day 1



Accuracy Study Day 2



Accuracy Study Day 3



Sensitivity and Stochastic Study Design



The following amounts of the positive control 007 were amplified, *in quadruplicate*, using the Globalfiler STR Kit to assess the sensitivity and stochastic effects of the GlobalFiler STR kit on the 3500 Genetic Analyzer:

15.625 pg, 31.25 pg, 62.5 pg, 125 pg, 250 pg, 500 pg, and 1 ng

The above samples were injected on the 3500 Genetic Analyzer using the standard injection parameters (1 μ l/15 secs).



Sensitivity and Stochastic Study

007 dilution series (43 total alleles)

ng/ μ l	pg/ μ l	input template	average # of alleles detected
0.1	100	1 ng	43
0.05	50	500 pg	43
0.025	25	250 pg	43
0.0125	12.5	125 pg	42
0.00625	6.25	62.5 pg	32.3
0.003125	3.125	31.25 pg	6.3
0.0015625	1.5625	15.625 pg	1

Sensitivity and Stochastic Study

* * * * * * * * * * * * * * *

	D3S1358	VWA	D16S539	CSF1PO	TPOX	Yindel	AMEL	D8S1179	D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045	D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338
1 ng-1	Green	Green	Green	Green	Grey	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
1 ng-2	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
1 ng-3	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
1 ng-4	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
500 pg-1	Green	Green	Green	Green	Grey	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
500 pg-2	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
500 pg-3	Green	Green	Green	Green	Grey	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green
500 pg-4	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
250 pg-1	Green	Green	Yellow	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
250 pg-2	Green	Green	Green	Green	Grey	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Yellow
250 pg-3	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Yellow	Green	Green
250 pg-4	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Green
125 pg-1	Yellow	Yellow	Green	Green	Grey	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Red	Green	Green	Green	Yellow
125 pg-2	Yellow	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Green
125 pg-3	Green	Green	Green	Green	Green	Green	Yellow	Green	Yellow	Green	Green	Green	Green	Red	Green	Green	Green	Green	Green	Green	Green	Yellow	Green	Red
125 pg-4	Yellow	Green	Yellow	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Red	Yellow	Green	Green	Green	Yellow
62.5 pg-1	Green	Red	Red	Red	Green	Yellow	Red	Red	Red	Red	Red	Red	Red	Red	Green	Green	Green	Green	Red	Red	Red	Red	Red	Red
62.5 pg-2	Green	Green	Green	Red	Green	Yellow	Yellow	Green	Green	Green	Green	Yellow	Red	Yellow	Yellow	Red	Green	Green	Green	Green	Yellow	Red	Red	Green
62.5 pg-3	Green	Yellow	Red	Yellow	Green	Green	Red	Green	Green	Green	Green	Green	Yellow	Red	Green	Yellow	Green	Green	Yellow	Green	Yellow	Green	Red	Red
62.5 pg-4	Red	Green	Green	Yellow	Green	Green	Yellow	Green	Red	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Red	Green	Red	Red	Red	Red
31.25 pg-1	Red	Red	Red	Red	Grey	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
31.25 pg-2	Green	Red	Red	Red	Grey	Red	Red	Red	Red	Red	Red	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
31.25 pg-3	Red	Red	Red	Red	Grey	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
31.25 pg-4	Red	Red	Red	Red	Grey	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red

- peak height ratio >70%
- peak height ratio < 70%
- allele drop-out
- homozygous at this locus
- * CODIS core loci

Mixture Study Design



The positive controls 9947A and 007 were mixed in the following ratios:

19:1, 9:1, 4:1, 3:2, 1:1, 1:0, 0:1, 1:1, 2:3, 1:4, 1:9, and 1:19

Approximately one nanogram of each mixture was amplified with the GlobalFiler STR Kit, in triplicate, and then injected on the 3500 Genetic Analyzer (*1 μ l/15 secs and 2 μ l/20 secs*).

Mixture Studies

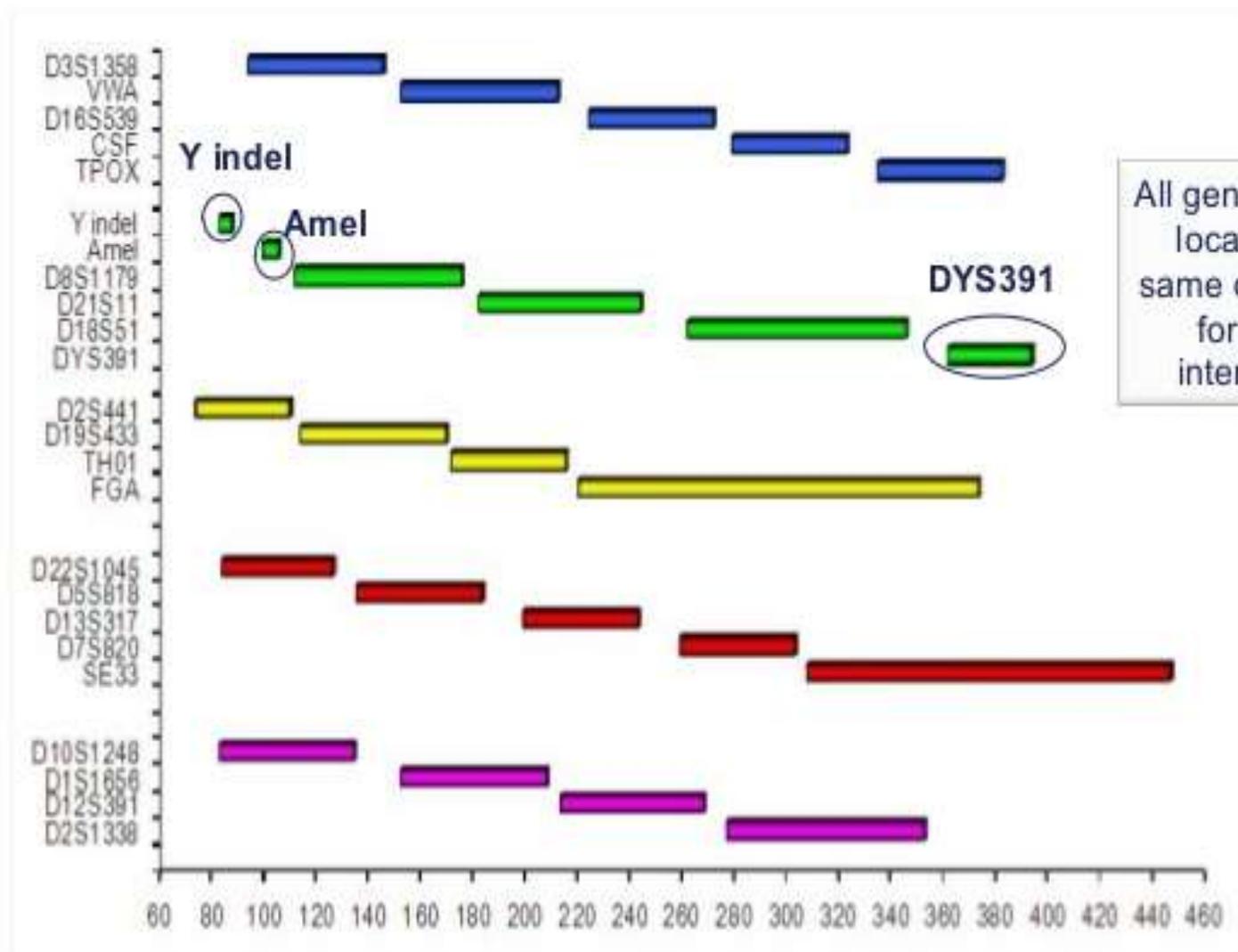
Standard injection parameters (1 μ l\15 secs)

	007 (26 unique alleles)		9947A (20 unique alleles)	
007:9947A	# 007 alleles detected	% complete 007	# 9947A alleles detected	% complete 9947A
19:1	26	100 %	13	65 %
9:1	26	100 %	19.7	98.5 %
4:1	26	100 %	20	100 %
3:2	26	100 %	20	100 %
1:1	26	100 %	20	100 %
2:3	26	100 %	20	100 %
1:4	26	100 %	20	100 %
1:9	24	92.3 %	20	100 %
1:19	16.3	62.7 %	20	100 %

Maximum injection parameters (2 μ l\20 secs)

	007 (26 unique alleles)		9947A (20 unique alleles)	
007:9947A	# 007 alleles detected	% complete 007	# 9947A alleles detected	% complete 9947A
19:1	26	100 %	17	85 %
9:1	26	100 %	20	100 %
1:9	24.7	95 %	20	100 %
1:19	20	76.9 %	20	100 %

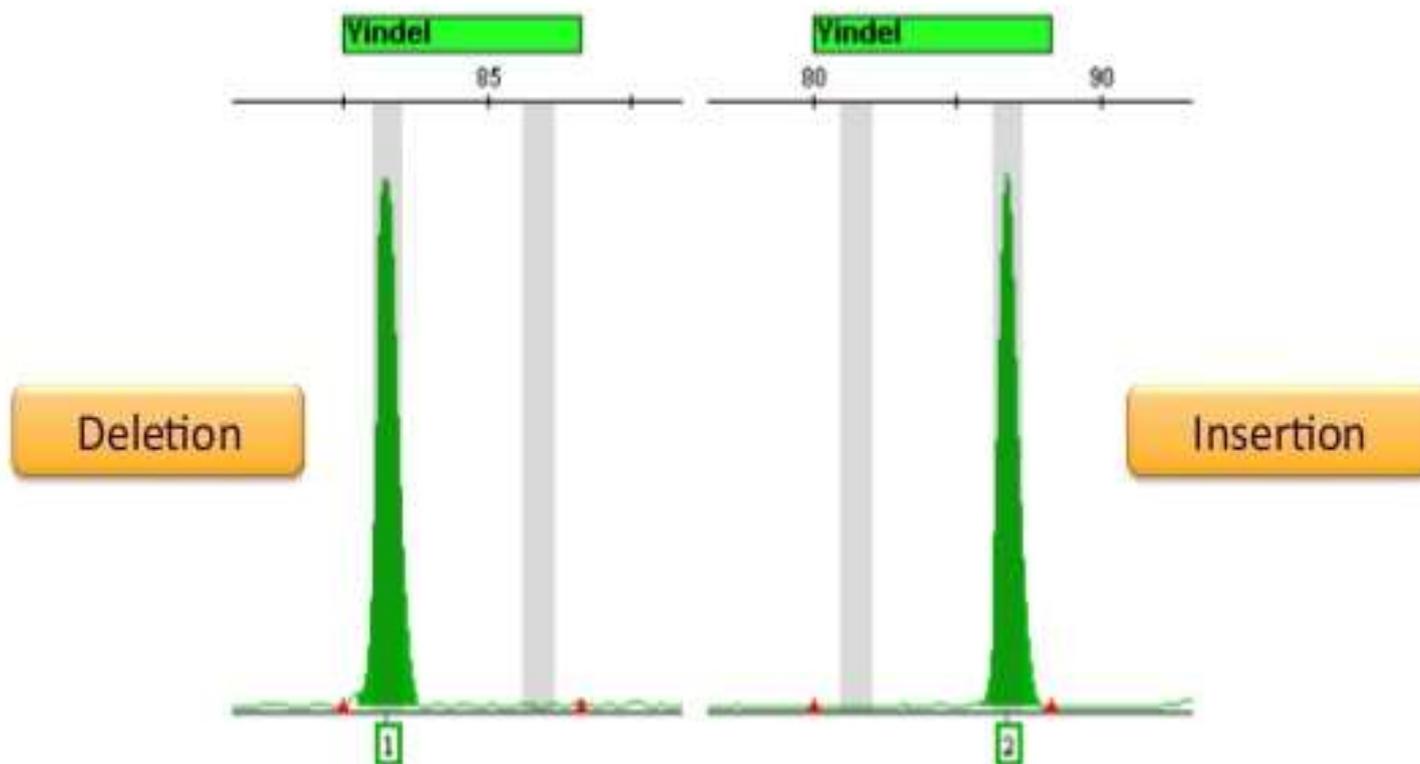
The GlobalFiler™ Kits: Configuration



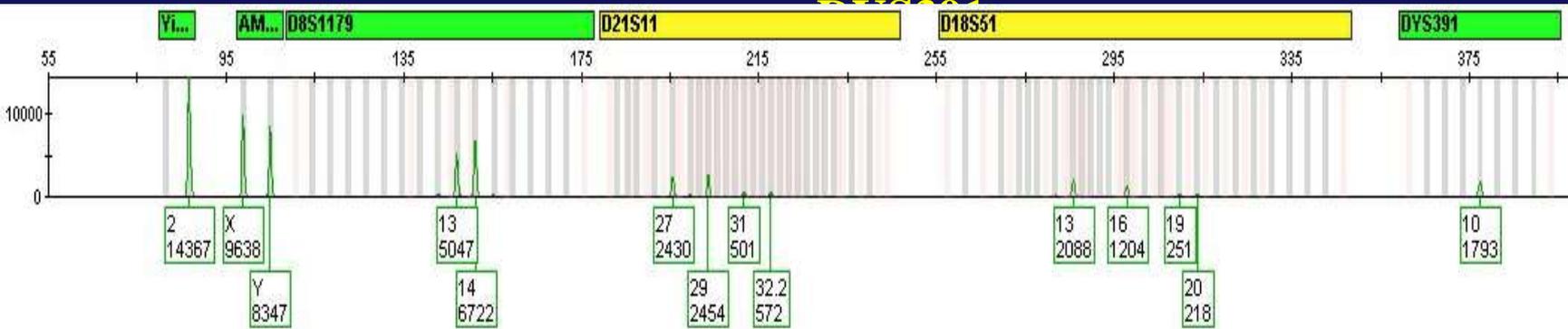
All gender markers located in the same dye channel for ease of interpretation

What is an Indel?

- Indels are short length polymorphisms, consisting of the presence (**IN**sertion) or absence (**DE**letion) of a short sequence (1-50 nucleotides)
- In the GlobalFiler™ Kits, an Indel marker on the Y chromosome was incorporated into the multiplex

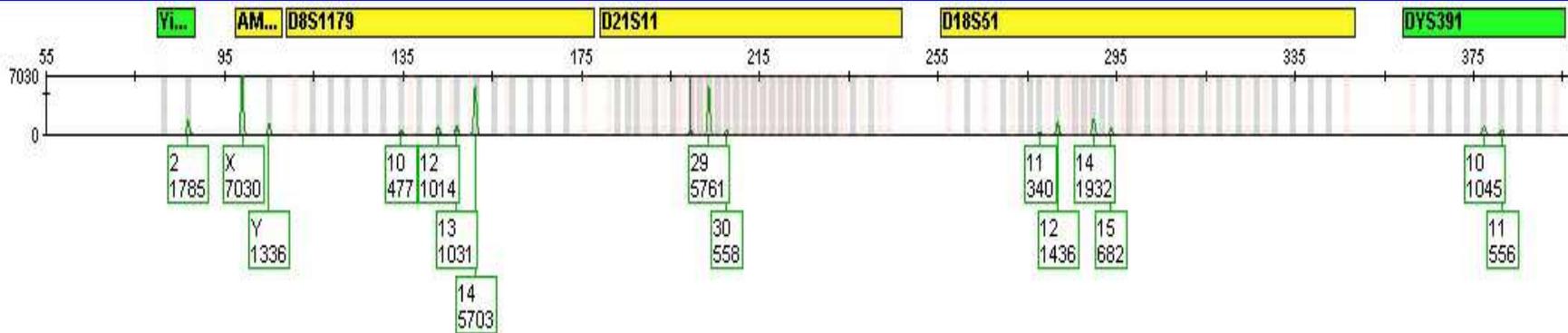


Mixture Studies



-mixture containing at least 1 male

10 allele

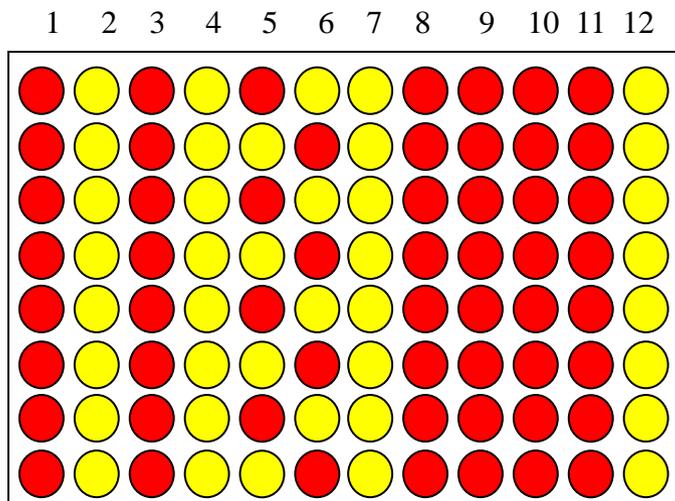


-mixture containing at least 2 males

10 and 11 alleles

Contamination Assessment Study Design

To evaluate the possibility of carry-over signal from injection to injection, a variety of configurations of excessive GS600-LIZ size standard (4X) and blank formamide were processed on the 3500



Injections occurred from left to right with single injections for columns 1-4.

Columns 5 and 6 injected three (3) times each consecutively.

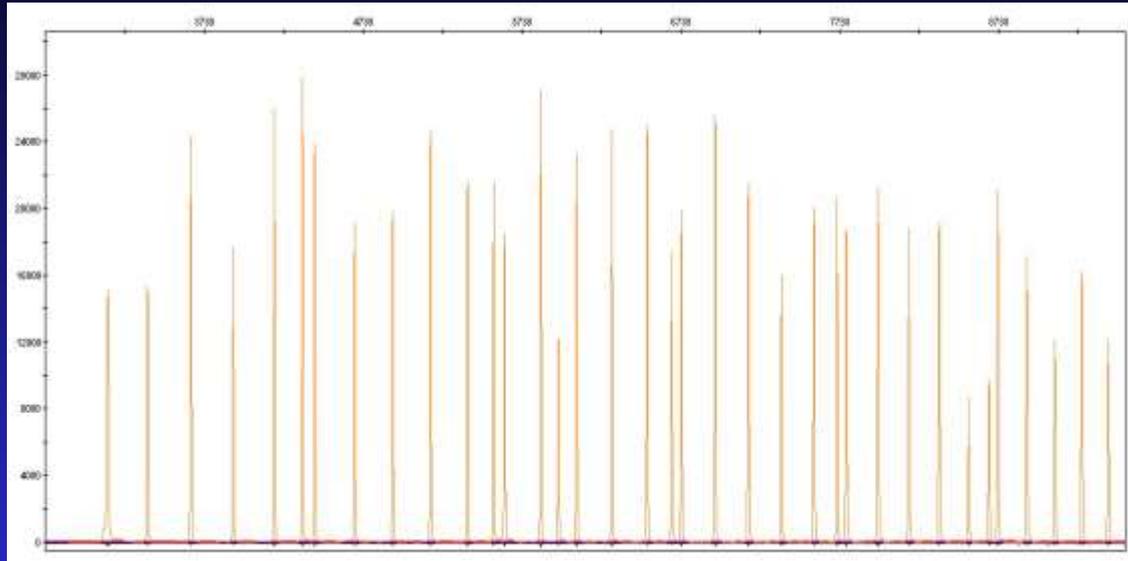
Single injections for columns 7-12.

Form only

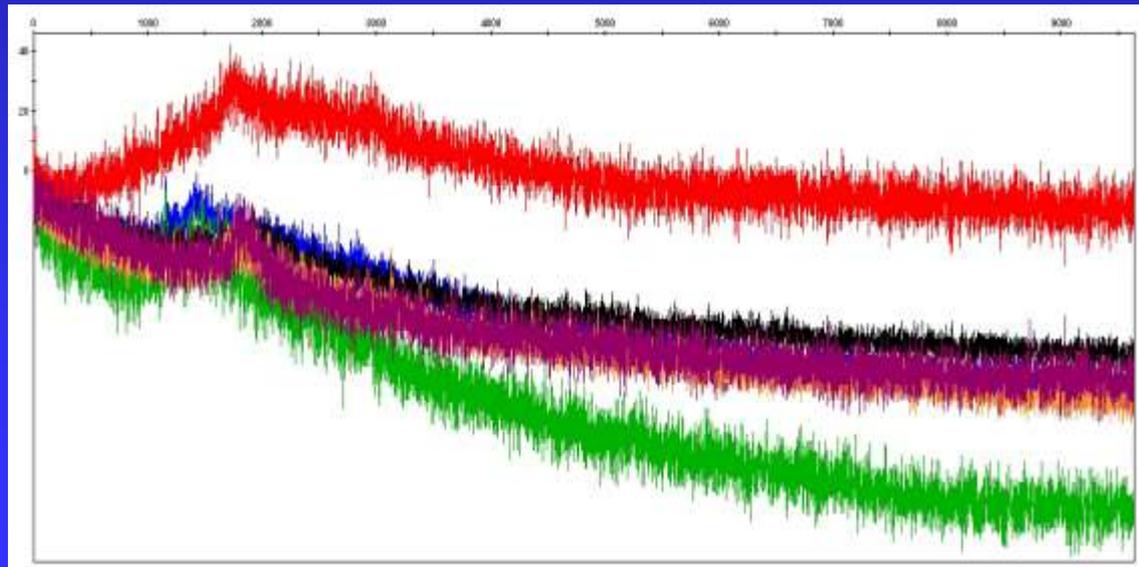
Form + ESS

Contamination Assessment

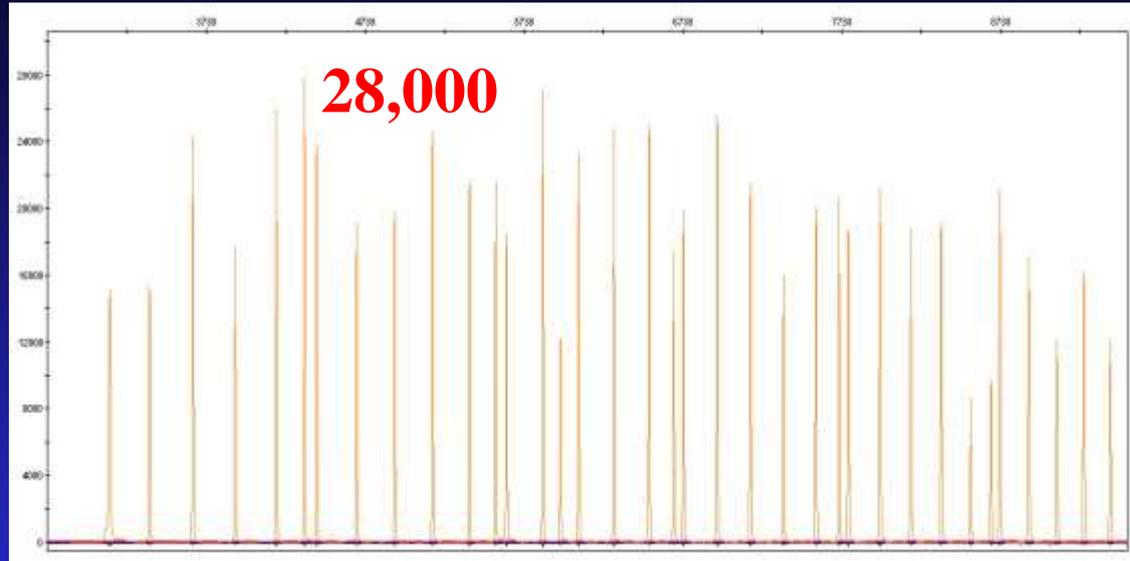
GS 600 LIZ



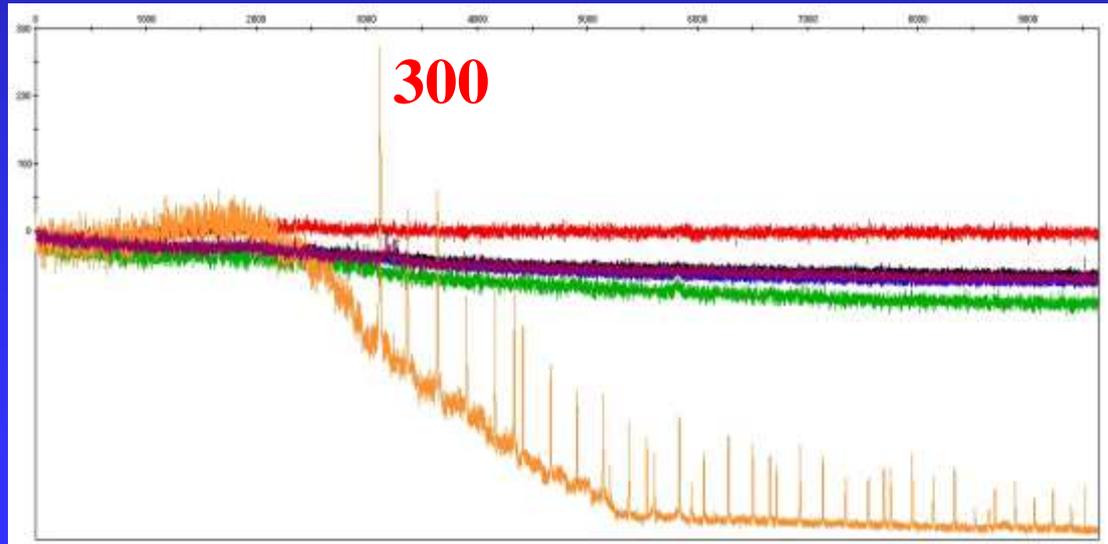
Blank



Contamination Assessment



GS 600 LIZ



Blank

Final Thoughts

- 1) **Reproducibility and precision of the GlobalFiler STR Kit on the ABI 3500:** the standard deviation across multiple allelic ladders injections was ≤ 0.05 bp.
- 2) **Accuracy of the GlobalFiler STR Kit on the ABI 3500:** with the exception of D8S1179, all alleles detected from the 007 sample across three separate 3500 runs were within ± 0.25 bp of the allelic ladder measurement. The alleles detected at D8S1179 were within ± 0.30 bp.
- 3) **Contamination assessment on the ABI 3500:** unexpected signal was detected in the formamide-only blanks – no carry-over detected in any amplification blanks employed in the rest of the validation studies.
- 4) **Mixture studies:** significant minor component could be detected in the 1:19 and 19:1 mixture samples.
- 5) **Sensitivity and stochastic studies:** full DNA profiles were obtained from 125 pg of template and significant data were obtained from 62.5 pg of template.
- 6) **Casework samples:** concordance between DNA profiles previously obtained with Identifiler. The GlobalFiler STR Kit was successful in overcoming both inhibition and degradation.

Acknowledgements

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Questions?





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